PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C07B 59/00	A1	WO 93/22260
		(43) International Publication Date: 11 November 1993 (11.11.93)

(21) International Application Number: PCT/GB93/00869

(22) International Filing Date: 27 April 1993 (27.04.93)

(30) Priority data:
92303905.1 30 April 1992 (30.04.92) EP
(34) Countries for which the regional or international application was filed: GB et al.

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: RADIOLABELLED COMPOUND FORMULATIONS

(57) Abstract

Additives are proposed for compositions comprising radiolabelled organic compounds e.g. 32P-labelled nucleotides. Stabilisers are selected from tryptophan, para-aminobenzoate, indoleacetate and the azole group. Dyes are selected from Sulphorhodamine B, Xylene Cyanol, Azocarmine B and New Coccine. Preferred compositions contain both stabiliser and dye.

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RADIOLABELLED COMPOUND FORMULATIONS

Radiolytic self-decomposition of radiochemicals has always been a problem to manufacturers and users alike. Shelf-life can be as little as a few weeks despite the use of the most suitable storage temperatures and physical dispersal methods for each particular compound or isotope. The subject is discussed in Review 16, Self-decomposition of Radiochemicals, Amersham International plc, Amersham.

Generally applicable additives which could be added to radiochemicals in order to extend shelf-life and improve efficiency by minimising the formation of radioactive impurities, would be of great economic and scientific value. A user of a stabilised radiochemical would benefit from being able to conduct experiments over a longer time span, achieve more consistent results between batches of the same radiochemical, and use less rigorous storage conditions. The additive should minimally interfere with or be compatible with the processes occurring in the application of radiochemicals to experimental systems, such as protein or nucleic acid manipulation.

US 4,390,517 teaches the use of a wide range of soluble primary, secondary and tertiary amines as stabilisers for radiolabelled compounds.

US 4,411,881 teaches the use of thiocarbonylated amines as stabilisers.

US 4,451,451 teaches the use of 4-aminobenzoic acid as an antioxidant in compositions containing Technetium-99m.

US 4,793,987 teaches the use of a range of

pyridine carboxylic acids as stabilisers.

32-P radiolabelled nucleotides are often sold as buffered aqueous solutions shipped in dry ice and sold for storage by the customer at -20°C. It would be a significant advantage, both to the shipper and to the customer, if the radiolabelled nucleotides could be supplied at ambient temperature and stored in an unfrozen form.

Solutions of radiolabelled nucleotides and other organic compounds are generally sold colourless. A coloured solution would be an advantage, since it would make the solution more easily visible during manipulation. However, a suitable dye would need not to interfere with any process in which the radiolabelled organic chemical might be used.

In one aspect the invention provides a composition comprising an organic compound labelled with a β-emitting radionuclide, said radiolabelled organic compound being subject to radiolytic decomposition during storage and shipment, together with a stabiliser selected from tryptophan, para-aminobenzoate, indoleacetate, luminol, and the group of azoles which are compounds having a 5-membered ring with at least two ring nitrogen atoms directly bonded to one another.

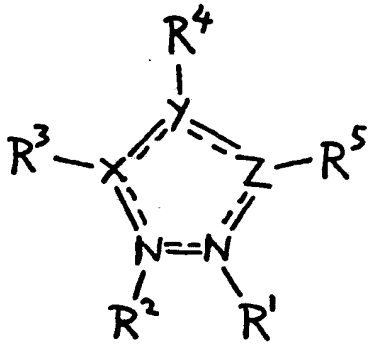
In another aspect, the invention provides a composition comprising a solution of an organic compound labelled with a β-emitting radionuclide and a dye.

The invention is mainly concerned with radiolabelled organic compounds which are supplied, shipped and stored in solution, usually aqueous solution or less usually in solution in a hydrophilic organic solvent. The invention also encompasses compositions in the solid state e.g. those produced by lyophilising or otherwise drying liquid compositions.

The invention is applicable to radiolabelled organic compounds which are subject to radiolytic self-decomposition, for example: amino acids, steroids, lipids, fatty acids, peptides, carbohydrates, proteins, and particularly nucleotides, thionucleotides, nucleosides and nucleic acids.

The nature of the β-emitting radionuclide is not critical; 3-H and 14-C are possible, but 32-P, 35-S and 33-P are preferred.

The stabiliser is preferably selected from L- and D-tryptophan; para-aminobenzoate which term is used to include the free acid and salts and esters thereof; indoleacetate which term is used to include the free acid and salts and esters thereof; luminol (3-aminophthalhydrazide); and the group of azoles which are compounds having a 5-membered ring with at least two ring nitrogen atoms directly bonded to one another. Such compounds preferably have the structure



which structure contains two ring double bonds, wherein

- one or two of X, Y and Z may represent N or one of X, Y and Z may represent S, the remaining X, Y and Z representing C,
- when present each of R^1, R^2, R^3, R^4 and R^5, represents -OH, -SH, -H, -COOH, $-NH_2$, $-CH_3$ attached

to the ring directly or via a chain of up to 10 carbon atoms, or two adjacent members of R^1, R^2, R^3, R^4 and R^5 may together constitute an aromatic ring.

It will be understood that R^1, R^2, R^3, R^4 and R^5 will or will not be present depending on the nature of X, Y and Z and on the positions of the two double bonds. Examples of classes of azole compounds envisaged are

X	Y	Z	Present	Absent
C	C	C	R^1, R^3, R^4, R^5	R^2
N	C	C	R^1, R^4, R^5	R^2, R^3
N	N	C	R^1, R^5	R^2, R^3, R^4
C	N	C	R^3, R^4, R^5	R^1, R^2
C	N	C	R^1, R^3, R^5	R^2, R^4
C	S	C	R^3, R^5	R^1, R^2, R^4

Among the possible compounds from the azole group are those illustrated in the Examples. The concentration of stabiliser is sufficient to reduce radiolytic decomposition of the radiolabelled organic compound, while not being so high as to materially interfere with the reaction systems where the radiolabelled organic compound is to be used. Preferred concentrations in liquid compositions are in the range of 1 mM to 1M, particularly 10 to 100 mM. Used in these concentrations, the preferred compounds have proved effective stabilisers particularly for nucleotides.

The dye is preferably selected from Sulphorhodamine B, Xylene Cyanol, Azocarmine B and New Coccine. Other possible dyes include Orange G,

Tartrazine, Safranin O, Methyl Green, Bromophenol Blue, Eosin, Evans Blue, Brilliant Blue G, Bromocresol Green, Ponceau S, Carmoisine Red, Remazol Red RB, Sandoz Black, Sandoz Violet, Sandoz Brilliant Green, Remazol Golden Yellow, Remazol Red B, Acid Red 40, Acid Alizarin Violet N, Mordant Brown 6 and BPBDTC (3,3´-(4,4´-biphenylene)-bis(2,5-diphenyl-2H-tetrazolium chloride). The concentration of the dye should be sufficient to visibly colour the solution, but not so high as to materially interfere with the reaction systems into which the radiolabelled organic compound is to be introduced. Preferred dye concentrations are from 20 to 3000 µg/ml, particularly 50 to 400 µg/ml; that is to say approximately (depending on the molecular weight of the dye) from 3×10^{-5} to 6×10^{-3} mol/l particularly 8×10^{-5} to 1×10^{-3} mol/l. At these concentrations, the dyes do have a mild stabilising effect, in addition to providing colour. However, the colour of compositions containing these dyes does fade with time, possibly due to radiolytic rupture of double bonds of the ring structures of the dyes. While this fading does not render the compositions unworkable, it may nevertheless be inconvenient. The structural formulae of the preferred stabilisers and dyes used in this invention are given in Tables 1 and 2 respectively.

According to a further and preferred aspect of the invention, the radiolabelled organic compound composition includes both the dye and the stabiliser. This has several advantages. The stabiliser helps to prevent the dye from fading. The dye improves the visibility of the radiochemical. The dye and the stabiliser may act synergistically to improve the stability of the radiolabelled organic compound.

The compositions of this invention may contain buffers. The nature of the buffer is not

critical to the invention, but standard commercial diluents for nucleotides consisting of an aqueous buffered solution stabilised by 2-mercaptoethanol or dithiothreitol are preferred systems. These are the systems that are used in the examples below. But other systems have been tested and shown to be equally effective.

Radiolabelled nucleotides and other organic compounds are conventionally shipped and stored at -20°C or below, requiring the use of dry ice. Preferred compositions according to this invention are suitable for shipment and storage either at 4°C (on ice) or more preferably at ambient temperature.

Experimental

In the examples below, various compositions were made up and tested for stability. Some of the tabulated experimental data refers to batches of dCTP labelled with 32 Phosphorus, but the stabilising compounds were also tested with the other 32 Phosphorus alpha-labelled nucleotides dATP, dGTP and dTTP. Testing of these stabilisers was also carried out with 32 Phosphorus gamma-labelled ATP and with 35 Sulphur alpha-labelled dATP. The half-life of 32P is 14.3 days, but batches for sale are typically reference-dated for the Friday of the week following sale. Stability testing was therefore carried out for 21 days to approximate the length of customer usage. Stabilisation of various compounds labelled with 3H, 14C and 33P was also investigated.

All test results are expressed as absolute percentage incorporation of the nucleotide compared with a control formulation, from the same batch, based on the above diluent without further added stabiliser or dye and stored at RT or +4°C or -20°C.

Various tests of nucleotide stability were

performed:

- The radiochemical purity of the labelled nucleotide was measured after storage for various intervals, using thin layer chromatography plates which were subsequently scanned using a Raytek RITA scanner. This is reported as RCP.

- Formulations were tested in various nucleic acid assays and manipulations: Sanger dideoxy sequencing using T7, Taq and Klenow DNA polymerase enzymes, random primed and nick translated DNA labelling reactions on both phage lambda and human genomic probes such as raf-1 and N-ras, and PCR labelling of probes. Probes generated as above were used in genomic hybridisations for single copy detection, and in colony screening. 3´ end tailing and 5´ end labelling of probes were also carried out, the latter specifically using the 32 Phosphorus gamma-labelled ATP.

Other techniques used were cDNA first strand synthesis and protein phosphorylation.

From these, random primed probe generation (in Amersham International Multiprime kit reactions: Amersham kit RPN 1600 based on Feinberg and Vogelstein, Anal. Biochem. 132, 6-13 (1983) and Addendum Anal. Biochem. 137, 266-267 (1984)) was selected as providing a stringent and representative test of radiolabelled organic compound stability and activity for the dNTPs: 5´end labelling was selected as the principal test for 32 P gamma-labelled ATP.

In the following examples, RCP refers to the radiochemical purity of the sample, MP to % incorporations obtained using the random primed DNA labelling technique in Amersham International´s Multiprime kit.

SB, XY, AB and NC are Sulphorhodamine B, Xylene Cyanol, Azocarmine B and New Coccine

respectively. pABA is para-aminobenzoate. IAA is indoleacetic acid. 2ME is 2-mercaptoethanol and DTT is dithiothreitol.

Storage conditions designated +40/RT/+4 indicate that the nucleotide was stored at +40°C for 24 hours, then at room temperature (RT; 21-24°C) for 48 hours before being stored at +4°C for the remainder of the test period.

Control samples consist of Amersham International's current selling nucleotide formulation, without the addition of any further stabiliser or dye.

EXAMPLES 1 TO 8

In Examples 1-8, the 32P labelled nucleotide (dCTP) was used at a specific activity of 3000 Ci/mmol and a concentration of 10 mCi/ml. 1 mCi lots were used for tests. Unless otherwise stated, the formulation used was an aqueous buffered diluent stabilised by 2-mercaptoethanol.

Example 1

Formulations containing different concentrations of stabilisers were tested. All stabilisers worked well.

SAMPLE	STORAGE	DAY 8		DAY 15		DAY 22	
		RCP	MP	RCP	MP	RCP	MP
CONTROL	+40/RT/+4	-	1	21	-	12	0
L-TRYPTOPHAN 25 mM	"	70	54	78	-	69	60
pABA Na 50 mM	"	79	73	77	-	79	66
pABA K 50 mM	"	80	71	82	-	79	70
IAA 50 mM	"	81	74	83	-	77	64

Example 2

Formulations containing the two dyes Sulphorhodamine B and Xylene Cyanol were made up and tested under different temperature storage conditions. Both dyes are seen to have a minor stabilising effect at +4°C.

SAMPLE	STORAGE	DAY 7		DAY 14		DAY 23	
		RCP	MP	RCP	MP	RCP	MP
CONTROL	-20°C	87	68	79	65	82	67
SB 400 µg/ml	"	87	61	81	65	84	69
XY "	"	77	60	73	64	74	60
CONTROL	+40/RT/+4	13	11	6	0	0	0
SB 400 µg/ml	"	35	35	15	9	0	0
XY "	"	29	30	16	5	0	0

Example 3

Formulations containing the stabiliser pABA K at the normal concentration of 50 mM and the dye New Coccine were tested. The dye was used at a final molarity of 3.5×10^{-4} mol/l (equivalent to Sulphorhodamine B at 200 µg/ml). Storage was at RT, 37°C or 42-45°C for either 1, 2 or 3 days as indicated, to test the robustness of the dye. After this period, all pots were stored at +4°C for the remainder of the test period.

SAMPLE	STORAGE	WK 0		WK 1		WK 2		WK3	
		RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL	-20°C	95	88	91	82	91	91	-	73
CONTROL	+40/RT/+4	89	80	94	57	95	66	-	12
NC+pABA K	72 HRS @ RT	92	75	93	71	76	76	-	-
"	24 HRS @ 37	97	72	91	82	82	83	-	74
"	48 HRS @ 37	92	64	92	83	91	82	-	64
"	72 HRS @ 37	79	73	94	83	94	79	-	-
"	24 HRS @ 42	89	75	94	81	90	79	-	36
"	48 HRS @ 42	92	81	93	76	91	69	-	-
"	72 HRS @ 42	96	77	77	81	91	76	-	-

Example 4

RCP's and % incorporations using the Multiprime assay were also measured for Azocarmine B, with experimental details as for Example 3. The dye was used at a final molarity of 3.5×10^{-4} mol/l.

SAMPLE	STORAGE	WK 0		WK 1		WK 2		WK 3	
		RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL	-20°C	93	89	88	82	93	91	-	71
CONTROL	+40/RT/+4	93	88	64	58	73	39	-	4
AB+pABA K	72 HRS @ RT	91	79	89	86	81	74	-	-
"	24 HRS @ 37	91	88	93	83	89	81	-	76
"	48 HRS @ 37	93	77	92	80	91	77	-	66
"	72 HRS @ 37	93	81	92	73	93	69	-	-
"	24 HRS @ 42	94	73	93	76	91	71	-	78
"	48 HRS @ 42	90	82	93	81	93	78	-	-
"	72 HRS @ 42	93	50	92	77	91	68	-	-

Example 5

Formulations containing two different dyes and two different stabilisers were tested. Both dyes were used at a concentration of 400 µg/ml. L-Tryptophan and potassium p-aminobenzoate were used at concentrations of 25 mM and 50 mM respectively.

SAMPLE	STORAGE	DAY 8		DAY 15		DAY 21	
		RCP	MP	RCP	MP	RCP	MP
CONTROL	+40/RT/+4	12	2	6	1	3	1
SB 400 µg/ml+L Tryp	"	71	48	66	49	61	34
SB 400 µg/ml+pABA K	"	76	52	67	53	66	41
XY 400 µg/ml+L Tryp	"	75	67	62	58	55	40
XY 400 µg/ml+pABA K	"	79	66	71	67	70	61

Example 6

A formulation containing 50 mM pABA K^+ was stored at RT, 37°C or 42-45°C for either 1, 2 or 3 days to test the robustness of the stabiliser.

All conditions except the control contain 50 mM pABA K.

After times at elevated temperatures as indicated, all conditions were stored at +4°C for the remaining test period except for the unstabilised control, which was kept at -20°C throughout.

SAMPLE	STORAGE	DAY 7		DAY 14	
		RCP	MP	RCP	MP
CONTROL	-20°C	89	85	86	78
pABA K	24 HRS @ RT	86	73	84	75
"	48 HRS @ RT	86	71	83	74
"	72 HRS @ RT	85	74	83	72
"	24 HRS @ 37	87	71	83	74
"	48 HRS @ 37	88	69	83	73
"	72 HRS @ 37	86	83	83	73
"	24 HRS @ 42	86	71	83	72
"	48 HRS @ 42	86	79	84	72
"	72 HRS @ 42	87	70	83	72

Example 7

Formulations containing different concentrations of Sulphorhodamine B as dye and para-amino Benzoic acid (Potassium salt) as stabiliser, and combinations of the two in various concentrations, were tested.

SAMPLE	STORAGE	DAY 7		DAY 15		DAY 22	
		RCP	MP	RCP	MP	RCP	MP
CONTROL	-20°C	84	74	67	67	75	63
20mM pABA K	+40/RT/+4	83	68	71	59	68	49
30 "	"	84	65	79	63	74	53
40 "	"	86	67	84	64	78	54
50 "	"	89	69	86	70	81	62
50 µg/ml SB	"	20	8	7	1	0	0
100 "	"	23	10	6	1	0	0
200 "	"	30	18	10	4	-	0
400 "	"	37	24	16	8	20	0
20mM pABAK//50µg/ml SB	"	73	60	68	57	66	51
" 100 "	"	74	62	71	57	66	52
" 200 "	"	73	56	69	51	62	51
" 400 "	"	78	65	77	54	67	54
30mM pABAK//50µg/ml SB	"	85	68	75	64	72	56
" 100 "	"	83	70	77	83	71	68
" 200 "	"	77	66	71	75	70	57
" 400 "	"	80	67	76	67	75	59
40mM pABAK//50µg/ml SB	"	79	68	78	63	79	60
" 100 "	"	84	65	78	62	77	58
" 200 "	"	86	67	78	63	77	59
" 400 "	"	86	67	84	65	79	63
50mM pABAK//50µg/ml SB	"	88	71	87	63	83	63
" 100 "	"	88	69	85	64	81	66
" 200 "	"	86	72	87	63	81	68
" 400 "	"	87	75	86	65	81	67

Example 8

Formulations containing different stabilisers were made up with and without 400 µg/ml of Sulphorhodamine B.

SAMPLE	STORAGE	DAY 8		DAY 15		DAY 22	
		RCP	MP	RCP	MP	RCP	MP
CONTROL	+40/RT/+4	-	1	21	-	12	0
SB 400µg/ml//50mM LTRYP	"	70	45	82	-	73	63
" //50mM pABANa	"	79	72	88	-	78	72
" //50mM pABAK	"	83	73	88	-	80	72
L TRYPTOPHAN ONLY	"	70	54	78	-	69	60
pABA Na ONLY	"	79	73	77	-	79	66
pABA K ONLY	"	80	71	82	-	79	70

Example 9

In the following data, the nucleotide used in testing was ^{35}S dATP at a concentration of 10 mCi/ml. All the stabilising compounds were used at a concentration of 50 mM, and were temperature cycled at +40°C for 24 hours and room temperature for 48 hours before long term storage at +4°C. All samples contained 20 mM Dithiothreitol (DTT).

SAMPLE	WK 2		WK 4		WK 8		WK 14	
	RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	92	72	91	74	91	77	87	61
3-Amino-5-mercaptotriazole	92	67	91	73	89	78	88	53
2-Amino-1,3,4-thiadiazole	87	72	84	74	81	74	75	61
2,5-Dimercapto-1,3,4-thiadiazole	92	62	90	80	90	77	87	47
4-Methyl-4H-1,2,4-triazole-3-thiol	91	66	88	79	91	72	83	43
3,5-Diamino-1,2,4-triazole	91	73	84	75	83	73	65	53
3-Amino pyrazole	88	73	87	70	85	78	71	53
5-Amino-1,3,4-thiadiazole-2-thiol	92	79	92	79	94	88	71	70
3-Amino-5-hydroxypyrazole	84	71	84	71	83	75	72	63
1H-1,2,4-triazole-3-thiol	91	70	91	73	89	81	90	78
5-Mercaptotriazole (Na^+) $2H_2O$	91	73	91	78	89	78	86	55
p-Amino benzoic acid (K^+)	79	60	81	69	66	66	58	40
5-Mercapto-1-tetrazole acetic acid (Na^+)	91	75	86	80	78	70	53	40
5-Mercapto-1-methyl tetrazole	89	73	84	65	78	70	65	37

Stabilisation was observed in all formulations.

Example 10

The stabilisers of Example 9 were also tested on 32P dCTP labelled nucleotide where they were again used at a working concentration of 50 mM. The radioactive concentration of the dCTP was 10 mCi/ml. All samples contained 5 mM 2-mercaptoethanol. Storage conditions were +40/RT/+4 except for the -20°C control.

SAMPLE	DAY 6		DAY 14		DAY 21	
	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	79	71	80	68	79	70
3-Amino-5-mercaptotriazole	77	70	76	68	76	66
pABA K^+	78	65	76	63	71	61
2-Amino-1,3,4-thiadiazole	73	60	65	58	68	51
2,5-Dimercapto-1,3,4-thiadiazole	79	74	78	67	79	65
4-Methyl-4H-1,2,4-triazole-3-thiol	78	75	78	77	79	69
3,5-Diamino-1,2,4-triazole	78	72	76	73	74	65
5-Mercapto-1-tetrazole acetic acid (Na^+)	81	69	73	68	73	65
5-Mercapto-1-methyl tetrazole	75	70	77	66	71	70
3-Amino pyrazole	76	73	76	65	74	63
5-Amino-1,3,4-thiadiazole-2-thiol	72	83	78	70	74	66
3-Amino-5-hydroxypyrazole	69	76	74	64	68	58
1H-1,2,4-triazole-3-thiol	76	72	78	72	78	64
5-Mercaptotriazole (Na^+) $2H_2O$	75	71	78	68	76	67

The results indicate that these compounds showed stabilising activity of nucleotides in solution.

Example 11

Further compounds were also tested on the 32P labelled dCTP nucleotides as for Example 10, and were again used at a working concentration of 50 mM (except for luminol which was used at a working concentration of 45 mM).

SAMPLE	DAY 8		DAY 16		DAY 23	
	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	90	50	88	52	83	49
pABA K^+	87	44	82	33	78	31
5-Methyl-1H-benzotriazole	80	40	71	39	67	48
3-Amino-4-pyrazole carboxylic acid	84	45	81	42	75	47
3-Amino-5-mercaptotriazole	87	41	88	40	82	47
Luminol	80	39	80	44	77	47

Example 12

Formulations containing stabiliser and/or dye were tested on dATP (alpha-35S) nucleotide solutions which were at 10 mCi/ml radioactive concentration. The labelling on the table shows the stabiliser and/or dye present in each sample including their respective concentrations. Storage conditions were +40/RT/+4 except for the -20°C control.

SAMPLE	WK 2		WK 4		WK 8		WK 14	
	RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	82	64	65	41	22	23	15	16
CONTROL +40/RT/+4	56	45	0	0	0	0	0	0
20 mM DTT	92	71	91	52	78	43	65	50
50 mM pABA (Na^+)	88	77	77	43	76	44	54	45
25 mM Tryptophan	86	70	78	50	59	34	41	38
200 µg/ml SB	64	56	17	7	0	0	0	0
50 mM pABA, 200 µg/ml SB	86	69	74	31	67	34	48	42
DTT, pABA, SB 20 mM, 50 mM, 200 µg/ml	91	67	88	37	86	49	79	49

These results show that DTT, pABA, Tryptophan and to a small extent SB, all stabilised the 35S labelled nucleotide. The possibility of dye and stabiliser combinations was demonstrated.

Example 13

Formulations containing stabilisers were tested on dATP (alpha-35S). All samples were pH 10.0. Radioactive concentration was 10 mCi/ml. The stabilisers present are indicated in the results table for each sample. Storage conditions were +40/RT/+4. AMT = 3-Amino-5-mercaptotriazole.

SAMPLE	WK 2		WK 4		WK 8		WK 14	
	RCP	MP	RCP	MP	RCP	MP	RCP	MP
20 mM DTT CONTROL -20°C	94	55	89	70	81	66	78	67
50 mM AMT	95	68	94	76	90	60	88	84
50 mM AMT, 50 mM DTT	93	52	94	73	92	62	91	83
50 mM AMT, 20 mM DTT	93	64	94	84	92	70	91	84
50 mM AMT, 100 mM 2ME	95	60	94	78	92	72	93	80
50 mM AMT, 40 mM 2ME	94	50	93	73	92	70	90	77
25 mM AMT	93	67	92	81	87	70	80	69
25 mM AMT, 50 mM DTT	96	44	94	78	92	68	90	77
25 mM AMT, 20 mM DTT	96	53	93	85	90	75	89	75
25 mM AMT, 100 mM 2ME	95	45	94	72	92	76	92	83
25 mM AMT, 40 mM 2ME	95	50	92	87	91	77	89	80

It can be deduced that the three stabilisers azole, DTT and 2-ME may be used in combination to achieve adequate stabilisation. Azole stabiliser may also be used with no other stabiliser present.

EXAMPLES 14 TO 21

Examples 14 to 21 show further testing of stabilisers on various radiolabelled compounds. Unless otherwise indicated, all stabilisers were used at a working concentration of 50 mM.

Example 14

Stabilisers were tested on dATP (alpha 35S) nucleotide solutions. All the samples were pH 10.0 and the radioactive concentration was 10 mCi/ml. All samples contained 20 mM DTT. Storage conditions were +40/RT/+4, except for the first two controls which were stored at -20°C.

SAMPLE	WK 2		WK 4		WK 8		WK 14	
	RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	95	61	92	76	90	82	90	76
CONTROL -20°C	93	51	91	78	87	80	87	76
CONTROL +40/RT/+4	92	53	58	56	40	39	30	38
5-Amino-1,3,4-thiadiazole-2-thiol	95	55	91	72	90	71	85	76
2-Amino-1,3,4-thiadiazole	92	56	88	71	85	71	85	72
4-Methyl-4H-1,2,4-triazole-3-thiol	91	49	88	63	87	64	91	68
3-Amino pyrazole	93	56	85	70	85	71	88	79
3,5-Diamino triazole	91	53	79	69	85	73	85	79

Example 15

A further experiment was carried out to test stabilisers on dATP (Alpha 35S). Experimental details were as for Example 14.

SAMPLE	WK 2		WK 4		WK 8		WK 14	
	RCP	MP	RCP	MP	RCP	MP	RCP	MP
CONTROL -20°C	93	67	90	74	88	72	89	76
CONTROL -20°C	93	65	90	76	87	77	84	74
CONTROL +40/RT/+4	90	61	83	77	75	71	62	60
5-Amino-1,3,4-thiadiazole-2-thiol	93	54	90	79	87	66	90	76
4-Methyl-4H-1,2,4-triazole-3-thiol	93	48	91	73	88	61	88	74
3-Amino pyrazole	91	64	87	78	83	70	80	80
3-Amino pyrazole-4-carboxylic acid	91	61	86	82	83	72	79	80
3,5-Diamino triazole	90	58	90	77	84	77	82	75
25 mM Tryptophan	90	51	90	74	--	70	79	74
3-Amino-5-mercapto triazole	92	55	91	73	85	74	88	73

Example 16

Stabilisers were tested on 33P gamma-labelled ATP. All samples contained 0.1% 2-mercaptoethanol. The radioactive concentration was 5 mCi/ml. All samples were stored at +4°C except the one control sample stored at -20°C, (there was no temperature cycling).

SAMPLE	DAY 6	DAY 14	DAY 45
	RCP	RCP	RCP
CONTROL -20°C	88	86	83
CONTROL +4°C	76	64	50
5-Amino-1,3,4-thiadiazole-2-thiol	90	89	87
4-Methyl-4H-1,2,4-triazole-3-thiol	90	87	86
3-Amino-5-hydroxypyrazole	89	81	78
3-Amino-5-mercaptotriazole	88	77	75
3,5-Diamino-1,2,4-triazole	89	88	81

All stabilisers showed a stabilisation effect, with all the purities being greater than those of the +4°C Control. The presence of some of the stabilisers maintained the purity of the nucleotide solution more effectively than storage at -20°C.

Example 17

Stabilisers were tested on 35S labelled methionine. All samples contained 0.1% 2-mercaptoethanol. The radioactive concentration was 34 mCi/ml. All samples were stored at +4°C except the first Control sample which was stored at -20°C.

SAMPLE	DAY 7	DAY 14	DAY 25	DAY 32
	RCP	RCP	RCP	RCP
CONTROL -20°C	84	63	41	27
CONTROL +4°C	52	10	3	--
5-Amino-1,3,4-thiadiazole-2-thiol	95	93	87	83
4-Methyl-4H-1,2,4-triazole-3-thiol	93	90	84	84
3-Amino-5-hydroxypyrazole	67	19	2	--
3-Amino-5-mercaptotriazole	94	94	92	92
3,5-Diamino-1,2,4-triazole	82	39	12	5

All stabilisers provided some stabilisation compared with the 4°C control. Several of these stabilisers conferred better stability on the 35S methionine at +4°C than storage at -20°C without the stabilisers present.

Example 18

Stabilisers were tested on ^{3}H labelled phenylalanine. The radioactive concentration was 0.5 mCi/ml. All samples were stored at room temperature except the first Control sample which was stored at +2°C.

SAMPLE	DAY 13	DAY 23	DAY 36	DAY 41
	RCP	RCP	RCP	RCP
CONTROL +2°C	82	74	74	68
CONTROL RT	81	71	69	62
5-Amino-1,3,4-thiadiazole-2-thiol	92	90	93	92
3,5-Diamino-1,2,4-triazole	91	87	87	86
3-Amino-5-hydroxypyrazole	92	88	93	92
Para-aminobenzoic acid	92	88	93	91

All stabilisers provided stabilisation compared with both control samples. Excellent stability was achieved even though storage was at room temperature.

Example 19

Stabilisers were tested on (Methyl-3H) Thymidine. The radioactive concentration was 0.5 mCi/ml. All samples were stored at room temperature except the first Control sample which was stored at +2°C.

SAMPLE	DAY 13	DAY 23	DAY 36	DAY 41
	RCP	RCP	RCP	RCP
CONTROL +2°C	--	74	69	68
CONTROL RT	78	73	66	64
5-Amino-1,3,4-thiadiazole-2-thiol	86	87	85	85
3,5-Diamino-1,2,4-triazole	85	84	84	85
3-Amino-5-hydroxypyrazole	86	83	85	86
Para-aminobenzoic acid	85	86	85	86

All stabilisers provided some stabilisation compared with both control samples. Excellent stability was achieved even though all stabilised samples were stored at room temperature.

Example 20

Stabilisers were tested on L-(U-14C) Histidine. The radioactive concentration was 100 mCi/ml. All samples were stored at room temperature except the first Control sample which was stored at +2°C.

SAMPLE	DAY 13	DAY 23	DAY 36	DAY 41
	RCP	RCP	RCP	RCP
CONTROL +2°C	99	99	98	99
CONTROL RT	97	97	96	95
5-Amino-1,3,4-thiadiazole-2-thiol	97	98	99	97
3,5-Diamino-1,2,4-triazole	98	97	98	98
Para-aminobenzoic acid	99	98	98	97

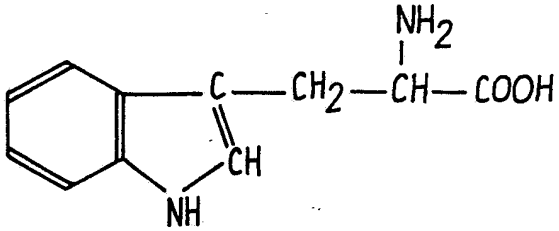
The stabilisers provided some stabilisation compared with the RT control sample. All samples performed well. The 14C half-life is very long (5730 years) and because of this, 14C-labelled compounds would be expected to be more stable. Long-term stability studies would be expected to show that the samples containing stabilisers have a significant stability improvement compared with controls.

Example 21

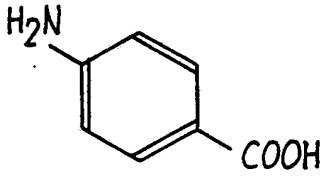
The stability of other compounds was determined in a similar manner. L-(5-3H) Proline (at 0.5 mCi/ml) and (8-14C) ATP (at 0.75 mCi/ml) were analysed over a period of six weeks. It was found that these compounds were quite stable, even with no stabiliser present. Both compounds maintained their purities at approximately 97-98%. From these results it can be concluded that the presence of the stabilisers does not reduce the stability of L-(5-3H) Proline and (8-14C) ATP.

TABLE.1.

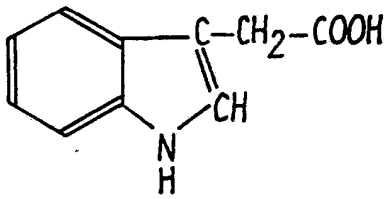
TRYPTOPHAN



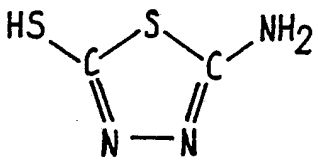
P-AMINOBENZOIC ACID



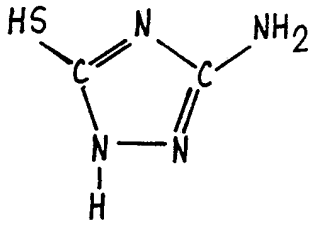
INDOLEACETIC ACID



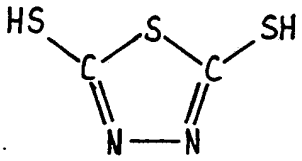
5- AMINO-1,3,4-THIADIAZOLE-2-THIOL



3- AMINO-5-MERCAPTO-1,2,4-TRIAZOLE



2,5-DIMERCAPTO-1,3,4-THIADIAZOLE



3,5-DIAMINO-1,2,4-TRIAZOLE

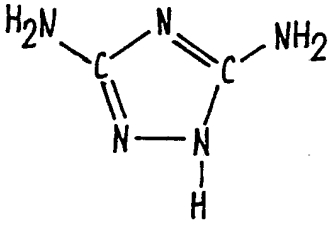
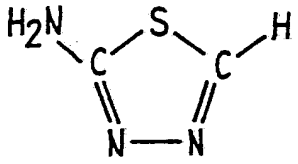
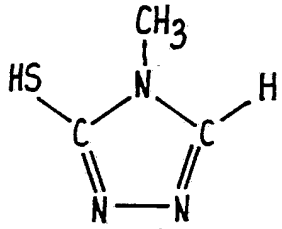


TABLE.1. CONTINUED 1

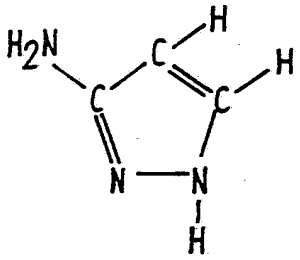
2- AMINO-1,3,4-THIADIAZOLE



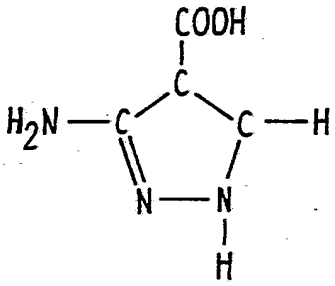
4- METHYL-4H-1,2,4-TRIAZOLE-3-THIOL



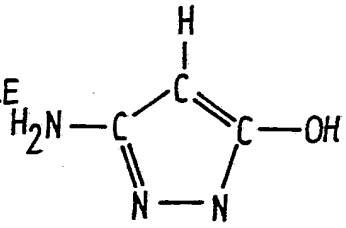
3-AMINO PYRAZOLE



3-AMINO-4- PYRAZOLE CARBOXYLIC ACID



3-AMINO-5-HYDROXY PYRAZOLE



4- AMINO-1,2,4 - TRIAZOLE

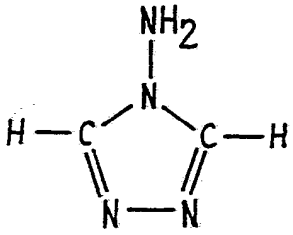
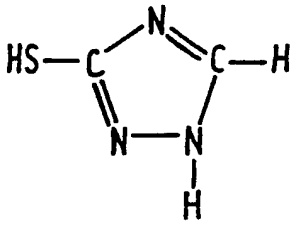
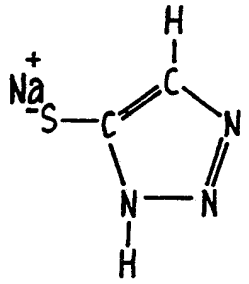


TABLE.1. CONTINUED. 2.

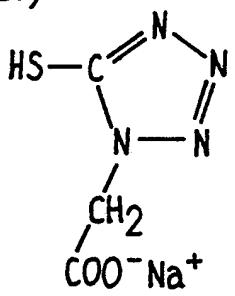
1H-1,2,4-TRIAZOLE-3-THIOL



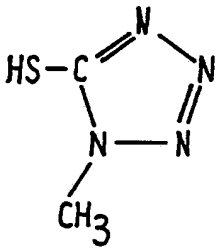
5-MERCAPTO-1,2,3-TRIAZOLE (Na^+ SALT)



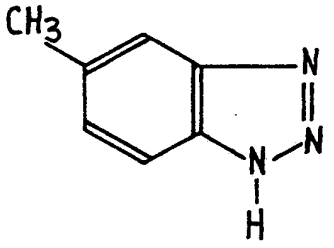
5-MERCAPTO-1-TETRAZOLEACETIC ACID (Na^+ SALT)



5-MERCAPTO-1-METHYL TETRAZOLE



5-METHYL-1H-BENZOTRIAZOLE



3-AMINOPHTHALHYDRAZIDE

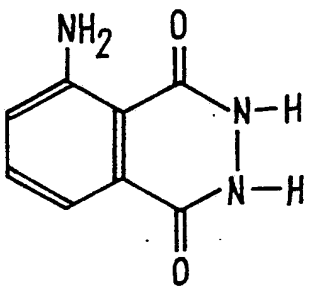
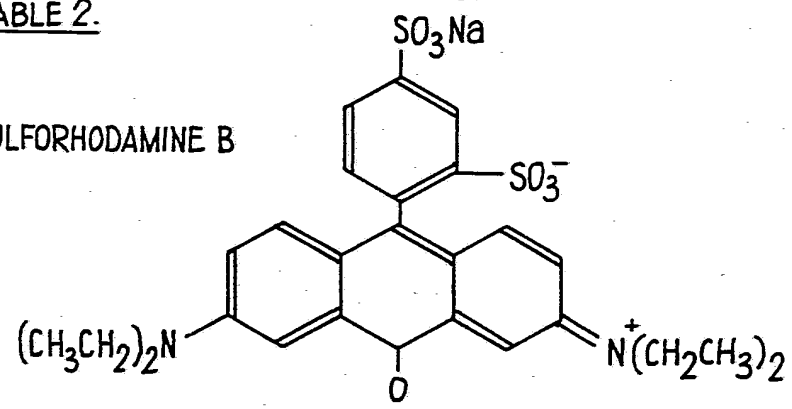
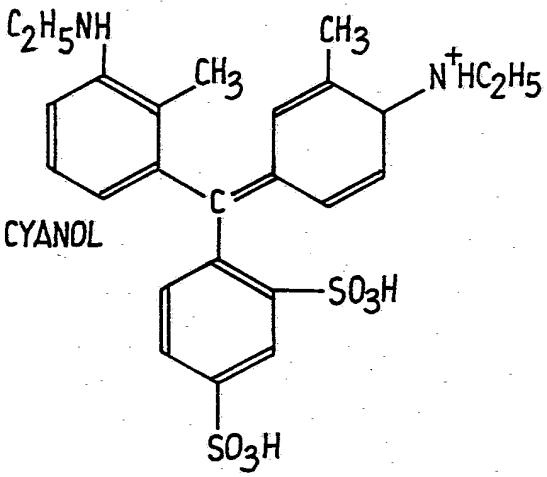


TABLE 2.

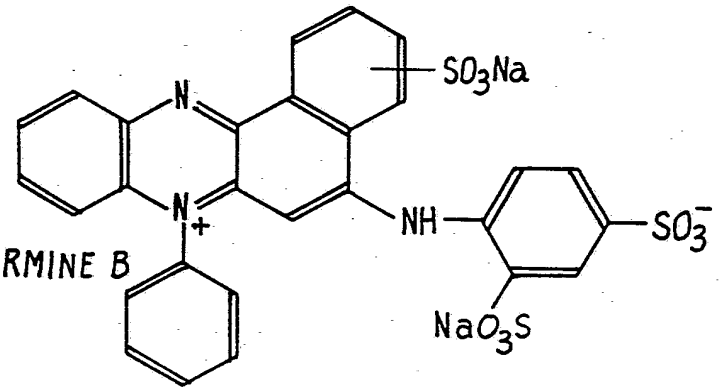
SULFORHODAMINE B



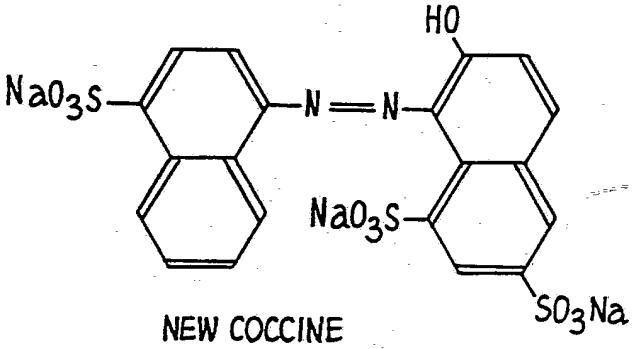
XYLENE CYANOL



AZOCARMINE B



NEW COCCINE



CLAIMS

1. A composition comprising an organic compound labelled with a β-emitting radionuclide, said radiolabelled organic compound being subject to radiolytic decomposition during storage and shipment, together with a stabiliser selected from tryptophan, para-aminobenzoate, indoleacetate, luminol, and the group of azoles which are compounds having a 5-membered ring with at least two ring nitrogen atoms directly bonded to one another.

2. A composition comprising a solution of an organic compound labelled with a β-emitting radionuclide and a dye.

3. A composition comprising a radiolabelled organic compound labelled with a β-emitting radionuclide, a dye, and a stabiliser selected from L-tryptophan, para-aminobenzoate, indoleacetate, luminol, and the group of azoles which are compounds having a 5-membered ring with at least two ring nitrogen atoms directly bonded to one another.

4. A composition as claimed in claim 1 or claim 3, wherein the radiolabelled organic compound is present in solution.

5. A composition as claimed in any one of claims 1 to 4, wherein the radiolabelled organic compound is a nucleotide.

6. A composition as claimed in any one of claims 1 to 4, wherein the radiolabelled organic compound is an amino acid.

7. A composition as claimed in any one of claims 1 to 6, wherein the radiolabel is selected from 32-P, 35-S, 33-P, 3-H and 14-C.

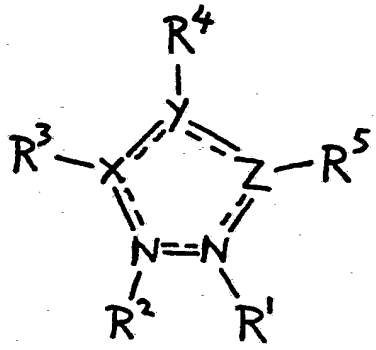
8. A composition as claimed in any one of claims 1 and 3 to 7, wherein the stabiliser is present at a concentration of 10 - 100 mM.

9. A composition as claimed in any one of claims 2 to 7, wherein the dye is present at a concentration of 50 - 400 µg/ml.

10. A composition as claimed in any one of claims 1 to 9, which is suitable for shipment and storage at 4°C or ambient temperature.

11. A composition as claimed in any one of claims 2 to 10, wherein the dye is selected from Sulphorhodamine B, Xylene Cyanol, Azocarmine B and New Coccine.

12. A composition as claimed in any one of claims 1 to 11, wherein the azole is one having the formula



which structure contains two ring double bonds, wherein

- one or two of X, Y and Z may represent N or one of X, Y and Z may represent S, the remaining X, Y and Z representing C,
- when present each of R^1, R^2, R^3, R^4 and R^5, represents -OH, -SH, -H, -COOH, $-NH_2$, $-CH_3$ attached to the ring directly or via a chain of up to 10 carbon atoms, or two adjacent members of R^1, R^2, R^3, R^4 and R^5 may together constitute an aromatic ring.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00869

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07B59/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07B ; G01N ; C12Q ; C07H A61K ; C09K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	US,A,4 451 451 (J. RIMMER) 29 May 1984 cited in the application see the whole document	1,3-10, 12
A	US,A,4 793 987 (A. HENDERSON) 27 December 1988 cited in the application see the whole document	1,3-10, 12
A	US,A,4 411 881 (N. R. TZODIKOV) 25 October 1983 cited in the application see the whole document	1,3-10, 12
A	US,A,4 390 517 (R. E. O'BRIEN) 28 June 1983 cited in the application see claims	1,3-10, 12

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 JULY 1993	28. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	WRIGHT M.W.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 111, no. 11, 11 September 1989, Columbus, Ohio, US; abstract no. 93463b, K. NAGAI 'APPARATUS FOR BASE SEQUENCE DETERMINATION IN NUCLEIC ACIDS' page 388 ;column 1 ; see abstract & JP,A,63 118 661 (HITACHI)	2,11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300869
SA 73152

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 22/07/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4451451	29-05-84	AU-B- 560024	26-03-87
		AU-A- 8988882	05-05-83
		CA-A- 1190473	16-07-85
		EP-A,B 0078642	11-05-83
		JP-C- 1364440	09-02-87
		JP-A- 58085823	23-05-83
		JP-B- 61032291	25-07-86
US-A-4793987	27-12-88	EP-A- 0203696	03-12-86
		JP-B- 4026712	08-05-92
		JP-A- 62000861	06-01-87
US-A-4411881	25-10-83	CA-A- 1205070	27-05-86
		CH-A- 655853	30-05-86
		DE-A,C 3324593	02-02-84
		FR-A,B 2536998	08-06-84
		GB-A,B 2123412	01-02-84
		JP-C- 1589949	30-11-90
		JP-B- 2011105	12-03-90
		JP-A- 59024257	07-02-84
US-A-4390517	28-06-83	US-A- 4358434	09-11-82
		CA-A- 1198365	24-12-85
		CA-C- 1198549	24-12-85
		EP-A,B 0031121	01-07-81
		JP-A- 2110113	23-04-90
		JP-B- 1005256	30-01-89
		JP-C- 1524438	12-10-89
		JP-A- 56097871	06-08-81